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小金梅草化学成分研究

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摘 要:从小金梅草乙醇提取物中分离得到了 12 个化合物,分别为 3-*O*-*β*-D-槲皮素葡萄糖苷(**1**)、3-*O*-*β*-D-山柰酚葡萄糖苷(**2**)、5-*O*-*β*-D-芹菜素葡萄糖苷(**3**)、α-菠甾醇(**4**)、2,6-二甲氧基苯甲酸(**5**)、3-吲哚甲酸(**6**)、(2*S*,3*R*,4*E*,8*E*)-1-(*β*-D-吡喃葡萄糖苷)-*N*-[(*R*)-2′-羟基-二十碳酰基]-9-甲基-4,8-二烯-1,3-二醇-2-氨基-十八烷(**7**)、正三十二烷醇(**8**)、14,15-二十碳烯酸(**9**)、木腊酸(**10**)、*β*-谷甾醇(**11**)、胡萝卜苷(**12**)。以上化合物均为首次从该植物中得到。

关键词:小金梅草; 黄酮; 化学成分

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Chemical Constituents from *Hypoxis aurea* Lour.

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Abstract: Twelve compounds were isolated from the ethanol extract of *Hypoxis aurea*, and their structures were elucidated by physicochemical properties and spectroscopic analysis as quercetin-3-*O*-*β*-D-glucoside(**1**), kaemferol-3-*O*-*β*-D-glucoside(**2**), apigenin-5-*O*-*β*-D-glucopyranoside(**3**), α-spinasterol(**4**), 2,6-dimethoxy-benzoic acid(**5**), 1H-indole-3-carboxylic acid(**6**), (2*S*,3*R*,4*E*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3-hydroxy-2-[(*R*)-2′-hydroxyeicosanoyl) amino]-9-methy-4,8-octadecadiene(**7**), n-dotriacontanol(**8**), 14,15-eicosenicacid(**9**), lignoceric acid(**10**), *β*-sitoster-ol(**11**), daucosterol(**12**). All these compounds were found in this plant for the first time.

Key words: *Hypoxis aurea*; flavonoids; chemical constituents

Introduction

Plants of the family Hypoxidaceae are widely distributed in Torrid Zone in the world, and previous studies of the family by our group revealed that it mainly contains phenolic glycosides and norlignans [1-3]. *Hypoxis aurea* Lour. belongs to the genus Hypoxidaceae, called as ´xiao jin mei´ to treat hernia and warm kidney in China [4] and mainly distributed over the southern part of China, southeast of Asia and Japan. Chemical investigation of the rhizomes of *H. aurea* toward potentially bioactive secondary metabolites from this genus leaded to the isolation of twelve compounds. These compounds were elucidated as quercetin-3-*O*-*β*-D-glucoside (**1**), kaemferol-3-*O*-*β*-D-glucoside (**2**), apigenin-5-*O*-*β*-D-glucopyranoside (**3**), α-spinasterol (**4**), 2,6-dimethoxy-benzoic acid (**5**), 1H-indole-3-carboxylic acid (**6**), (2*S*,3*R*,4*E*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3-hydroxy-2-[(*R*)-2-hydroxyeicosanoly) amino]-9-methy-4,8-octadecadiene (**7**), n-dotriacontanol (**8**), 14,15-eicosenic acid (**9**), lignoceric acid (**10**), *β*-sitosterol (**11**) and daucosterol (**12**) on the basis of spectroscopic analysis and comparing spectral data with those known compounds reported in literatures. All these compounds were found in this plant for the first time.

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Experimental

General

Melting points were measured on a XRC-1 micro-melting point apparatus and were uncorrected. MS spectra were obtained on a VG Auto Spec-3000 mass spectrometer. ^{1}H and ^{13}C (DEPT) NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 MHz spectrometers, with chemical shifts (δ) in ppm relative to TMS as internal standard and coupling constants in hertz (Hz). Silica gel (200-300 mesh) for column chromatography was product of the Qingdao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Reversed-phase chromatography was with RP-18 (LiChroprep, 40-63 μm, Merck, Darmstadt, Germany).

Plant materials

The whole plant of *H. aurea*. were collected in Kunming, Yunnan Province, People's Republic of China, in September 2008, and authenticated by professor Peng Hua. A voucher specimen (KUN 0864822) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered rhizomes of *H. aurea* (3.0 kg) were extracted three times each with 15 L of 95% EtOH under reflux for 3h. The extracts were evaporated and the residue was resuspended in 15 L of H_2O and partitioned successively with petroleum ether (3 L × 3), EtOAc (3 L × 3) and n-BuOH (3 L × 3) to yield petroleum ether extract (49 g), EtOAc extract (70 g), *n*-BuOH extract (210 g), respectively. The n-BuOH (200 g) extract was applied to a silica gel column chromatography (200-300 mesh) eluted with $CHCl_3$/CH_3OH/H_2O (15∶3∶0.5, v/v) to give five fractions. Fraction 3 (30 g) was purified by column chromatography silica gel with $CHCl_3$/CH_3OH/H_2 (7∶3∶0.5, v/v), to yield four sub-fractions based on TLC analysis. Sub-fraction 2, were purified on RP-18 with 20% to 100% aqueous CH_3OH, and on Sephadex LH-20 with CH_3OH/CH_3Cl (1∶1, v/v) to afford compounds **1** (12 mg), **2** (8 mg) and **3** (5 mg). EtOAc extract (60 g) was subjected to column chromatography on silica gel (200-300 mesh) column eluted with petroleum ether/acetone (10∶1, v/v) to give five fractions. Sub-fraction 3 (35 g) was repeatedly purified over silica gel (200-300 mesh) eluted with $CHCl_3$/MeOH (from 20∶1 to 5∶1, v/v), and then on Sephadex LH-20 eluted with MeOH/$CHCl_3$ (1∶1, v/v) to yield compounds **4** (14 mg), **5** (10 mg), **6** (5 mg), **7** (15mg), **10** (18 mg), **11** (146 mg) and **12** (76 mg). Sub-fraction **1** (19 g) was chromatographyed on silica gel (200-300 mesh) using petroleum ether/acetone (20∶1, v/v) as an eluant to give the compounds **8** (258 mg) and **9** (35 mg).

Results and Discussion

Quercetin-3-*O*-β-D-glucoside (1) Yellow powder, $C_{21}H_{20}O_{12}$, mp. 242-245 ℃, FABMS (-) *m/z*: 463 $[M-H]^-$, ^{1}H NMR (CD_3OD, 400 MHz) δ: 12.62 (1H, s, OH), 7.66 (1H, t, *J* = 8.4, 2.1 Hz, H-6′), 7.50 (1H, d, *J* = 2.1 Hz, H-2′), 6.80 (1H, d, *J* = 8.4 Hz, H-5′), 6.39 (1H, d, *J* = 2.0 Hz, H-6), 6.18 (1H, d, *J* = 2.0 Hz, H-8), 5.36 (1H, d, *J* = 7.7 Hz, H-1″), 3.15 ~ 3.63 (11H, m, H-2″ ~ 6″). ^{13}C NMR (CD_3OD, 100 MHz) δ: 156.3 (C-2), 133.4 (C-3), 177.5 (C-4), 161.3 (C-5), 98.7 (C-6), 164.2 (C-7), 93.6 (C-8), 156.3 (C-9), 103.9 (C-10), 121.1 (C-1′), 122.4 (C-2′), 156.2 (C-3′), 148.4 (C-4′), 115.9 (C-5′), 122.0 (C-6′), 101.3 (C-1″), 71.2 (C-2″), 75.9 (C-3″), 67.9 (C-4″), 73.2 (C-5″), 60.2 (C-6″). The MS and NMR data were identical to those of literature [5].

Kaemferol-3-*O*-β-D-glucoside (2) Yellow powder, $C_{21}H_{20}O_{11}$, mp. 178-179 ℃, FABMS (-) *m/z*: 447 $[M-H]^-$, ^{1}H NMR (CD_3OD, 500 MHz) δ: 8.07 (2H, d, *J* = 8.9 Hz, H-2′, 6′), 6.9 (2H, d, *J* = 8.9 Hz, H-3′, 5′), 6.32 (1H, d, *J* = 1.8 Hz, H-8), 6.14 (1H, d, *J* = 1.8 Hz, H-6), 5.08 (1H, d, *J* = 7.7 Hz, H-1″), 3.81 ~ 3.30 (11H, m, H-2″ ~ 6″). ^{13}C NMR (CD_3OD, 125 MHz) δ: 158.7 (C-2), 132.3 (C-3), 179.3 (C-4), 161.6 (C-5), 95.3 (C-6), 168.6 (C-7), 93.3 (C-8), 158.3 (C-9), 105.4 (C-10), 122.8 (C-1′), 135.5 (C-2′), 116.1 (C-3′), 158.7 (C-4′), 116.1 (C-5′), 132.3 (C-6′), 105.2 (C-1″), 73.1 (C-

2″), 77.1 (C-3″), 70.0 (C-4″), 75.1 (C-5″), 62.0 (C-6″). The MS and NMR data were identical to those of literature [5].

Apigenin-5-*O*-β-D-glucopyranoside (3) Yellow powder, $C_{21}H_{20}O_{10}$, mp. 240-242 ℃, FABMS (-), *m/z*: 431 [M-H]$^-$. ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.10 (2H, d, *J* = 8.8 Hz, H-2′,6′), 7.9 (1H, s, H-3), 6.9 (2H, d, *J* = 8.8 Hz, H-3′,5′), 6.41 (1H, d, *J* = 1.9 Hz, H-8), 6.21 (1H, d, *J* = 1.9 Hz, H-6), 5.15 (1H, d, *J* = 7.8 Hz, H-1″), 3.31 ~ 3.82 (m) protons of glucose; ^{13}C NMR (CD_3OD, 100 MHz) δ: 162.5 (C-2), 105.8 (C-3), 179.7 (C-4), 158.5 (C-5), 104.9 (C-6), 161.6 (C-7), 99.9 (C-8), 158.3 (C-9), 108.5 (C-10), 122.7 (1′), 128.1 (C-2′), 116.1 (C-3′), 160.9 (C-4′), 116.1 (C-5′), 128.5 (C-6′), 104.9 (C-1″), 73.0 (C-2″), 77.1 (C-3″), 67.0 (C-4″), 75.0 (C-5″), 61.9 (C-6″). The MS and NMR data were identical to those of literature [6].

α-Spinasterol (4) Colorless powder, $C_{29}H_{52}O_3$, mp. 250-252 ℃, EIMS *m/z*: 448 [M]$^+$, 430, 412, 397; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.82 (3H, s, 19-CH_3), 0.83 (3H, d, *J* = 8.6 Hz), 0.84 (3H, d, *J* = 6.3 Hz), 0.86 (3H, d, *J* = 7.5 Hz), 0.92 (3H, d, *J* = 6.6 Hz), 1.19 (3H, s, 18-CH_3), 4.07 (1H, m, H-3), 3.52 (1H, br s, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 31.2 (C-1), 32.7 (C-2), 67.8 (C-3), 41.1 (C-4), 76.3 (C-5), 76.4 (C-6), 34.3 (C-7), 28.4 (C-8), 46.2 (C-9), 38.7 (C-10), 21.4 (C-11), 40.3 (C-12), 43.1 (C-13), 56.6 (C-14), 24.4 (C-15), 26.8 (C-16), 56.3 (C-17), 12.1 (C-18), 17.0 (C-19), 36.4 (C-20), 19.3 (C-21), 34.9 (C-22), 26.8 (C-23), 46.3 (C-24), 29.7 (C-25), 18.9 (C-26), 19.9 (C-27), 23.5 (C-28), 12.3 (C-29). The 1H and ^{13}C NMR data were identical to those of literature [7].

2,6-Dimethoxy-benzoic acid (5) Colourless powder, $C_9H_{10}O_4$, mp. 164-165 ℃, FABMS (+) *m/z*: 183 [M+1]$^+$, 165 (M$^+$-OH), 150 (165-CH_3), 137 (M$^+$-COOH), 151 (M$^+$-OCH_3); ^{1}H NMR (CD_3OD, 500 MHz) δ: 3.80 (6H, s, 2OCH_3), 6.64 (2H, d, *J* = 8.4 Hz, H-3, H-5), 7.22 (1H, d, *J* = 8.4 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz) δ: 157.7 (C-2, C-6), 130.2 (C-4), 115.3 (C-1), 105.2 (C-3, C-5), 56.3 (2, 6-OMe). The MS and NMR spectral data were in consistent with those reported [8].

1H-indole-3-carboxylic acid (6) Yellow powder, $C_9H_7O_2N$, mp. 210-215 ℃, EIMS *m/z*: 161 [M]$^+$ (100), 144 (98), 116 (26), 89 (16). ^{1}H NMR (CD_3OD, 400 MHz) δ: 13.0 (1H, br s, N-H), 8.88 (1H, d, *J* = 7.8 Hz, H-7), 8.53 (1H, d, *J* = 2.7 Hz, H-2), 7.61 (1H, d, *J* = 8.0 Hz, H-4), 7.40 (1H, t, *J* = 7.8 Hz, H-6), 7.32 (1H, t, *J* = 7.8 Hz, H-5). ^{13}C NMR (CD_3OD, 100 MHz) δ: 133.0 (C-2), 108.7 (C-3), 122.0 (C-4), 123.6 (C-5), 122.4 (C-6), 112.9 (C-7), 138.2 (C-8), 127.5 (C-9), 169.3 (COOH). The MS and NMR data were identical to those of literature [9].

(2*S*, 3*R*, 4*E*, 8*E*)-1-(β-D-Glucopyranosyloxy)-3-hydroxy-2-[(*R*)-2-hydroxyeicosanoly) amino]-9-methy-4, 8-octadecadiene (7) Colorless powder, $C_{45}H_{85}NO_9$, mp. 190-192 ℃ FABMS (*m/z*) (%) 784 [M+H]$^+$ (15), 516 (6), 280 (11). ^{1}H NMR (C_5D_5N, 500 MHz) δ: 8.36 (1H, d, *J* = 9.4 Hz, NH), 4.95-4.19 (protons of glucose), 0.85 (3H, t, *J* = 6.9 Hz, H-18 or 21′). ^{13}C NMR (C_5D_5N, 125 MHz) δ: 71.5 (C-1), 54.6 (C-2), 72.3 (C-3), 132.1 (C-4), 132.2 (C-5), 130.2 (C-8), 131.1 (C-9), 32.9 (C-10), 29.6-30.1 (CH_2), 14.3 (C-18), 175.7 (C-1′), 72.5 (C-2′), 14.3 (C-5′), 105.7 (C-1″), 75.2 (C-2″), 78.5 (C-3″), 71.5 (C-4″), 78.6 (C-5″), 62.6 (C-6″). The NMR data were identical to those of literature [10].

1-Dotriacontanol (8) White powder, $C_{32}H_{66}O$, mp. 88-89 ℃, EIMS *m/z*: 448 [M-H_2O]$^+$, 420 (6), 392 (10), 139 (13). ^{1}H NMR ($CDCl_3$, 500 MHZ) δ: 0.88 (3H, t, *J* = 6.5 Hz), 1.26 ~ 1.30 (58H, br m), 1.60 (2H, m), 3.64 (2H, t, *J* = 7.0 Hz, CH_2OH); ^{13}C NMR ($CDCl_3$, 125 MHZ) δ: 64.4 (C-1), 34.4 (C-2), 31.9 ~ 22.7 (C-3 ~ 31), 14.1 (C-32). The MS and NMR data were identical to those of literature [11].

14,15-Eicosenic acid (9) White solid, $C_{20}H_{36}O_2$, mp. 158-159 ℃, EIMS *m/z*: 308 [M]$^+$, 290 [M-H_2O]$^+$, 266 (6), 239 (10). ^{1}H NMR ($CDCl_3$, 400 MHZ) δ: 0.88 (3H, t, *J* = 6.5 Hz), 1.26 ~ 4.30

(30H, br m), 5.24 (2H, m). The MS and ^{1}H NMR data were identical to those of literature [12].

Lignoceric acid (10) White solid, $C_{24}H_{48}O_2$, mp. 72-73 ℃, EIMS *m/z*: 368 $[M]^+$. ^{1}H NMR ($CDCl_3$, 400 MHZ) δ: 0.87 (3H, t, J = 6.7 Hz), 1.27-1.31 (40H, br m), 1.60 (2H, m), 2.44 (2H, t, J = 7.0, CH_2OH). The MS and NMR data were identical to those of literature [13]. Compounds **11** and **12** were determined to be β-sitosterol and daucostero respectively by comparing their TLC behaviors with standard samples.

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